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CLASSICAL AND ALTERNATE PATHWAY ACTIVATION OF COMPLEMENT IN PEMPHIGUS VULGARIS LESIONS*

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ABSTRACT

Early oral and skin lesions from 6 patients with pemphigus vulgaris were examined for the presence of C1q, C3, C3 proactivator (C3PA), and properdin, in addition to immunoglobulins (IgG, IgA, and IgM), using immunofluorescent techniques. IgG was deposited in the intercellular substance in both normal-appearing areas of the lesions and pathologic areas of the lesions. C1q, C3, and C3PA, on the other hand, were deposited only in pathologic areas where acantholysis was evident. Properdin deposits were evident in 2 lesions only, and then only around individual acantholytic cells. IgA localization was seen in intercellular substance areas only in oral lesions. These studies further implicate the complement system in the pathogenesis of pemphigus vulgaris.

Pemphigus vulgaris, characterized immunopathologically by autoantibodies to an intercellular substance (ICS) of skin and mucosa [1], may be a bullous skin disease mediated by the complement system. These ICS antibodies do not appear capable of complement fixation by in vitro complement staining methodology [2]. Nevertheless, by using direct immunofluorescent (IF) staining methods [3–5], C3 and more recently C1q and C4 [5], in addition to IgG, have been demonstrated in early, fresh pemphigus lesions. More recently, low levels of total hemolytic complement and the first 5 hemolytic complement components were found in pemphigus blister fluids, when compared to corresponding serum complement levels and other serum and blister fluid proteins [6]. All these studies suggest local activation of the classical complement pathway in pemphigus lesions and blister fluids.

Using immunofluorescent techniques, we designed the present studies to determine whether factors associated with the alternate or properdin pathway are present in ICS areas of pemphigus lesions in addition to components of the classical pathway.

PATIENTS AND METHODS

Six patients who had early pemphigus were included in this study. Oral lesions were obtained from 4 patients while skin lesions were obtained from the other 2. Biopsies from all 6 demonstrated suprabasal intraepidermal bulla formation with "acantholysis" consistent with pemphigus. Serum samples and biopsies of lesions were obtained from patients by standard procedures and were stored at −70°C until used. Oral and skin lesions from patients with other oral and skin diseases served as controls.

Antiserum to IgG was prepared, assayed, and conjugated with fluorescein isothiocyanate by methods previously described [7]. Antisera to IgA, IgM, and C3 (β_1C/β_1A globulins) were purchased (Hyland Division, Travenol Laboratories, Inc., Costa Mesa, Calif.) and tested for specificity by immunodiffusion and immunoelectrophoresis. Characteristics of these conjugated antisera appear in Table I.

Antiserum to C1q was prepared in rabbits according to Morse and Christian [8] and was used at a 1:100 dilution in the immunofluorescent tests. Rabbit antiserum to C3 proactivator (C3PA) was prepared and assayed by the methodology of Götze and Müller-Eberhard [9]. This antiserum was used at a 1:20 dilution. Antiserum to human properdin was made in a goat after isolating properdin according to Pensky, Hinz, Todd, Wedgwood, Boyer, and Lepow [10]. For immunofluorescent testing, this antiserum was used at a 1:10 dilution.

Patients' sera were tested for ICS-reactive antibodies by the indirect immunofluorescent staining method according to established procedures [7], using monkey esophageal mucosa sections. Direct immunofluorescent staining of biopsy specimens using antisera to IgG, IgA, IgM, and C3 was also performed by standard methodology [7].

A modified indirect immunofluorescent procedure, recently described [11], was used to test for the presence of C1q, C3PA, and properdin. Briefly, sections of lesions treated with unlabeled rabbit anti-C1q and rabbit anti-C3PA were then treated with labeled goat antirabbit IgG (F/P ratio 4.4). Labeled rabbit antigoat IgG (F/P ratio 3.5) was used as the second step of the procedure when lesion sections were treated initially with unlabeled goat antiproperdin. The labeled antisera

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TABLE I

Characteristics of labeled antihuman antisera

Antiserum	F/P ratio*	Unitage†	Use dilution
Anti-IgG	3.4	8	1:32
Anti-IgA	3.2	8	1:32
Anti-IgM	3.9	4	1:16
Anti-C3	3.5	8	1:40

* Molar fluorescein to protein ratio.

† Unitage determinations were performed in gel diffusion (Ouchterlony) by standard methods [7].

were purchased (Hyland) and were diluted 1:32 for the modified indirect immunofluorescent procedure.

Specificity controls for the above C3PA and properdin IF staining reactions included treating the tissues alone with the conjugated antirabbit and antigoat antisera, prior treatment with normal goat and rabbit sera followed by the conjugated antisera, and specific absorption of the unconjugated antisera (to C3PA and properdin) with C3PA and properdin, respectively.

RESULTS

A summary of immunofluorescent testing of 4 oral lesions and 2 skin lesions from 6 patients with early pemphigus appears in Table II. IgG was bound to the ICS areas in all 6 lesions, a finding consistent with many previous observations [1–5, 7]. IgA, in addition to IgG, was present in the 4 oral lesions but not in the skin lesions, again in the ICS areas. The intensity of the IgA staining was greatest in acantholytic areas. IgM was also noted in 2 of the early oral lesions.

ICS deposits of C3 were also apparent in all 6 of the early pemphigus lesions (Fig. 1A). These findings also support earlier studies [3–5]. ICS deposits of C1q, a subunit of the first complement component in the classical sequence, were present in 4 of 6 lesions. Like the IgA localization in oral pemphigus lesions, both C3 and C1q deposits occurred only in areas of acantholysis or newly forming areas of acantholysis.

Interestingly, C3PA deposits were found in 5 of the 6 pemphigus lesions (Fig. 1B). The intensity of C3PA staining, however, was considerably less than the intensity of C1q and C3 staining. C3PA deposition also was present only in areas where acantholysis was evident.

Properdin, on the other hand, was seen in only 2 specimens (Fig. 2). ICS staining occurred only in acantholytic areas and only around newly formed acantholytic cells. In contrast to C1q, C3, and C3PA, properdin was not present in early developing acantholytic areas.

Similar localization of immunoglobulins (IgG, IgA, and IgM), complement components (C1q and C3), C3PA, and properdin to the ICS areas was not seen in 80 skin biopsies of patients with various other dermatoses and 37 oral mucosa biopsies of patients with various types of oral pathology. This latter group consisted of 5 cases of cicatricial pemphigoid, 6 cases of oral lichen planus (Fig. 3), 8 cases of recurrent aphthous stomatitis, 4 cases of desquamative gingivitis, 3 cases of oral bullous pemphigoid, 1 case each of oral Sjögren's syndrome, Behçet's disease, and lupus erythematosus, and 8 cases of undiagnosed blisters of the oral cavity. Linear basement membrane zone deposition of C1q, C3, C3PA, and properdin seen in bullous pemphigoid lesions and previously noted by Provost and Tomasi [11] will be reported in the very near future [12].

ICS staining was not observed with treatment of pemphigus sections with conjugated goat antirabbit (Fig. 1C) or rabbit antigoat serum alone. Prior treatment of pemphigus lesions with normal goat or rabbit serum followed by conjugated antisera did not result in ICS staining. Finally, specific absorption of anti-C3PA with C3PA in 3 cases (cases 1, 2, and 3, Table II) and absorption of antiproperdin with properdin in 1 case (case 2, Table II) resulted in blocking of the specific IF staining reactions. Similar specificity controls, carried out on bullous pemphigoid lesions, which exhibit heavy properdin staining, also resulted in similar blocking when antiproperdin was absorbed with properdin [12].

DISCUSSION

The findings reported herein support recent immunofluorescent observations of van Joost, Cormane, and Pondman [5] and hemolytic complement component studies reported by Jordon, Day, Luckasen, and Good [6] suggesting local activation of the classical pathway in pemphigus vulgaris. Additional support includes the identification of an "anticomplementary" factor in pemphigus blis-

TABLE II

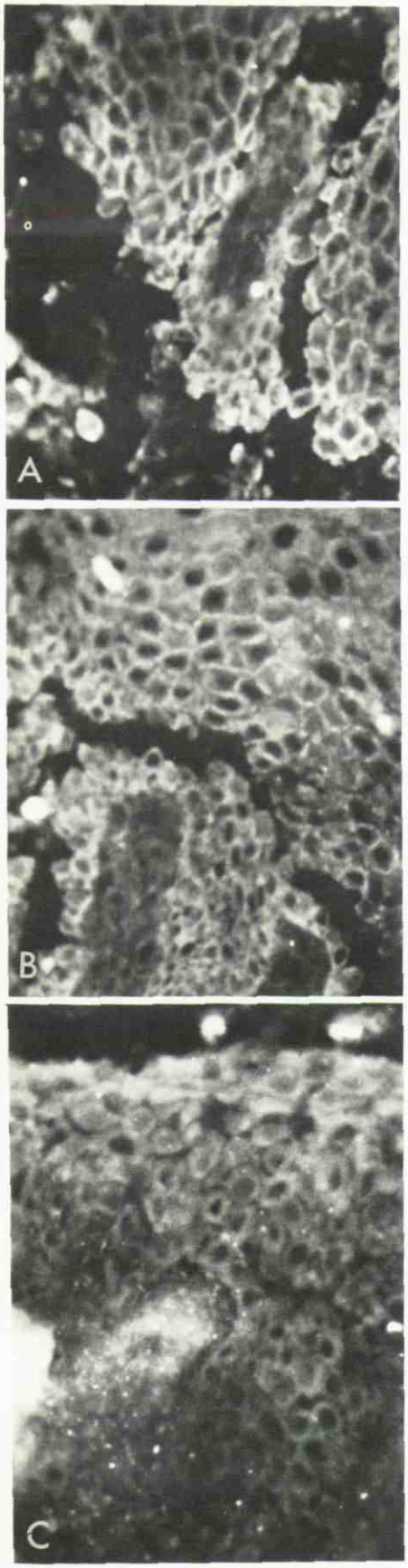
Summary of immunofluorescent studies of pemphigus vulgaris lesions

Antisera	Patients					
	1*	2*	3	4	5*	6*
Anti-IgG	+	+	+	+	+	+
Anti-IgA	+	+	–	–	+	+
Anti-IgM	–	+	–	–	+	–
Anti-C1q†	+	+	–	+	–	+
Anti-C3	+	+	+	+	+	+
Anti-C3PA†	+	+	+	–	+	+
Anti-properdin†	–	+	–	–	–	+
IIF titer§	1:320	Neg.	1:80	1:640	Neg.	1:160

* Oral lesions.

† Modified indirect immunofluorescent staining employed.

§ Indirect immunofluorescent titer of serum antibodies. "Neg." means no serum antibodies present.



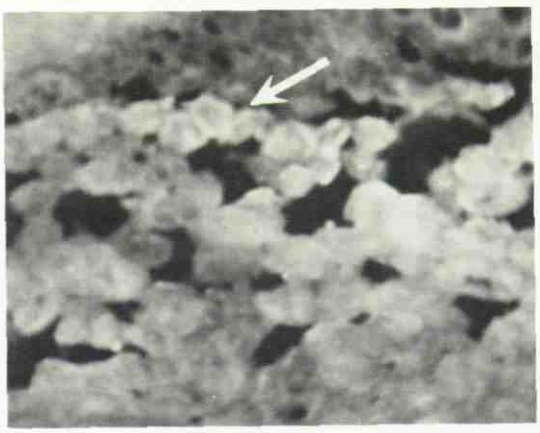


FIG. 2: Modified indirect IF staining demonstrating properdin deposition only around newly formed acantholytic cells (arrow) in an early mucosa lesion (× 216).

ter fluid which activates both early and late components in normal human serum [6]. This anticomplementary factor, now thought to represent an immune complex (unpublished observations), is still under investigation.

Significant activation of the alternate or properdin pathway, however, is questionable. Properdin, now thought to be the initiating factor of the alternate pathway [13], was present in only 2 pemphigus lesions, and only around acantholytic cells. Interestingly, these deposits were seen in oral mucosa lesions with heavy IgA deposits in addition to IgG. Two other oral lesions, however, did not reveal properdin deposition despite the fact that IgA was present.

It may be that the sensitivity of our properdin staining method is too low to detect such activity in pemphigus lesions. In bullous pemphigoid [12] and herpes gestationis [14], however, basement membrane zone deposition of properdin is readily detectable using the same reagents. Perhaps properdin is present in pemphigus lesions in only trace amounts and is undetectable using immunofluorescence. If this is the case then more sensitive serologic methods will have to be devised.

C3PA, on the other hand, was present in 5 of 6 pemphigus biopsies studied thus far. Since properdin was not present in all of these biopsies, the C3PA deposition might best be explained by the "C3 feedback mechanism." With activation of C3 by the classical pathway, "C3b" forms, which also activates C3PA through C3PA convertase [15, 16]. In addition to activating early complement components and C3, the blister-fluid anticomplementary

FIG. 1: Immunofluorescent (IF) staining of early lesions of pemphigus vulgaris. A. Direct IF staining demonstrating intercellular substance (ICS) deposition of C3 in early acantholytic areas of a skin lesion (× 235). B. Modified indirect IF staining demonstrating ICS deposition of C3PA in an oral mucosa lesion. Although less intense than C3, C3PA is again apparent only in acantholytic areas (× 235). C. Direct IF staining using goat antirabbit IgG antiserum. No ICS staining is apparent (× 235).

FIG. 3: Direct IF staining of an oral lesion from a patient with lichen planus using an antiserum to C3. No ICS staining is apparent (× 250).

factor described above also activates C3PA, a phenomenon also best explained by this C3 feedback mechanism [6].

If the classical complement pathway is the primary pathway involved in pemphigus vulgaris, the absence of C1q in 2 of the lesions must be explained further. Possibly, the C1 molecule dissociates from the complex once C4 and C2 are activated, as suggested by Rapp and Borsos [17]. Perhaps with further sectioning of these lesions, significant C1q binding might have become apparent. Similar findings, however, have been noted in *bullous pemphigoid lesions [11], where the absence* of C1q has been observed in the presence of C4 and C3 deposition. Hemolytic complement studies of bullous pemphigoid blister fluids also indicate classical pathway activation in that disease [18].

Our negative studies demonstrating the noncomplement-fixing activity of ICS antibodies, using in *vitro complement staining [2], are also somewhat* disturbing. It is important to note, however, that complement deposits in pemphigus lesions occur only in areas of acantholysis. IgG deposits, on the other hand, are more diffuse and occur in uninvolved adjacent areas in addition to pathologic areas. Possibly only in areas of lesion formation —acantholytic areas—are the ICS antibodies so arranged that complement activation can take place. What role complement plays in such areas, however, must await further investigation.

Our studies, however, suggest that acantholysis, the basic pathologic process in pemphigus, may be *mediated by the complement system.*

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REFERENCES

1. Beutner EH, Jordon RE: Demonstration of skin antibodies in sera of pemphigus vulgaris patients by indirect immunofluorescent staining. Proc Soc Exp Biol Med 117:505–510, 1964
2. Jordon RE, Sams WM Jr, Diaz G, Beutner EH: Negative complement immunofluorescence in pemphigus. J Invest Dermatol 57:407–410, 1971
3. Cormane RH, Chorzelski TP: "Bound" complement in the epidermis of patients with pemphigus vulgaris. Dermatologica 134:463–466, 1967
4. Cram DL, Fukuyama K: Immunohistochemistry of ultraviolet-induced pemphigus and pemphigoid lesions. Arch Dermatol 106:819–824, 1972
5. Van Joost T, Cormane RH, Pondman KW: Direct immunofluorescent study of the skin on occurrence of complement in pemphigus. Br J Dermatol 87:466–474, 1972
6. Jordon RE, Day NK, Luckasen JR, Good RA: Complement activation in pemphigus vulgaris blister fluid. Clin Exp Immunol 15:53–63, 1973
7. Beutner EH, Chorzelski TP, Jordon RE: Autosensitization, *in* Pemphigus and Bullous Pemphigoid. Springfield, Ill, Charles C Thomas, 1970
8. Morse JH, Christian CL: Immunological studies of the 11S protein component of the human complement system. J Exp Med 119:195–209, 1964
9. Götze O, Müller-Eberhard HJ: The C-3 activator system: an alternate pathway of complement activation. J Exp Med 134:90s–108s, 1971
10. Pensky J, Hinz CF Jr, Todd EW, Wedgwood RJ, Boyer JT, Lepow IH: Properties of highly purified human properdin. J Immunol 100:142–158, 1968
11. Provost TT, Tomasi TB Jr: Evidence for complement activation via the alternate pathway in skin diseases. I. Herpes gestationis, systemic lupus erythematosus, and bullous pemphigoid. J Clin Invest 52:1779–1787, 1973
12. Jordon RE, Schroeter AL, Good RA, Day NK: The complement system in bullous pemphigoid. II. Immunofluorescent evidence for both classical and alternate pathway activation. Clin Immunol Immunopathol (in press)
13. Götze O, Müller-Eberhard HJ: The role of properdin in the alternate pathway of complement activation. J Exp Med 139:44–57, 1974
14. Bushkell LL, Jordon RE, Goltz RW: Herpes gestationis: new immunologic findings. Arch Dermatol (in press)
15. Müller-Eberhard HJ, Götze O: C3 proactivator convertase and its mode of action. J Exp Med 135:1003–1008, 1972
16. Lachmann PJ, Nicol P: Reaction mechanism of the alternative pathway of complement fixation. Lancet 1:465–467, 1973
17. Rapp HJ, Borsos T: Molecular Basis of Complement Action. New York, Appleton-Century-Crofts, 1970, pp 23–35
18. Jordon RE, Day NK, Sams WM Jr, Good RA: The complement system in bullous pemphigoid. I. Complement and component levels in sera and blister fluids. J Clin Invest 52:1207–1214, 1973